

Starving our Microbial Self: The Deleterious Consequences of a Diet Deficient in Microbiota-Accessible Carbohydrates

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<http://dx.doi.org/10.1016/j.cmet.2014.07.003>

The gut microbiota of a healthy person may not be equivalent to a healthy microbiota. It is possible that the Western microbiota is actually dysbiotic and predisposes individuals to a variety of diseases. The asymmetric plasticity between the relatively stable human genome and the more malleable gut microbiome suggests that incompatibilities between the two could rapidly arise. The Western lifestyle, which includes a diet low in microbiota-accessible carbohydrates (MACs), has selected for a microbiota with altered membership and functionality compared to those of groups living traditional lifestyles. Interactions between resident microbes and host leading to immune dysregulation may explain several diseases that share inflammation as a common basis. The low-MAC Western diet results in poor production of gut microbiota-generated short-chain fatty acids (SCFAs), which attenuate inflammation through a variety of mechanisms in mouse models. Studies focused on modern and traditional societies, combined with animal models, are needed to characterize the connection between diet, microbiota composition, and function. Differentiating between an optimal microbiota, one that increases disease risk, and one that is causative or potentiates disease will be required to further understand both the etiology and possible treatments for health problems related to microbiota dysbiosis.

The Gut Microbiota: A Control Center for Human Biology

The human gut harbors a diverse ecosystem of trillions of microbial cells (Costello et al., 2012; Qin et al., 2010). The traditional view that gut microbiota effects are limited to the host's digestive tract, or more recently extended to metabolism and immune status, has given way to the realization that these microbes can have widespread impact on diverse aspects of a host's physiology (Sommer and Backhed, 2013). Externally applied forces, such as diet or antibiotics, result in rapid alterations of the microbiota, thereby affecting the status of host-microbiota relations (David et al., 2014; Dethlefsen et al., 2008; Dethlefsen and Relman, 2010; Walker et al., 2011). The malleability of our gut residents is further reframing this community's role as a platform for rational manipulation of host biology: the microbiota provides a manipulatable lever to improve human health and to treat or prevent disease (Sonnenburg and Fischbach, 2011; van Nood et al., 2013; Vrieze et al., 2012).

At a more fundamental level, the very plasticity that makes the gut microbiota an attractive therapeutic avenue can result in unintentional and maladaptive changes, or dysbiosis. A plethora of microbiota enumeration studies in recent years have implicated microbiota dysbiosis in a growing list of Western diseases, such as metabolic syndrome, inflammatory bowel disease, and cancer. These studies rely on comparing the microbiota of individuals that are diseased to those that are healthy. The contribution of the dysbiotic microbiota to disease phenotype has been illustrated via microbiota transplant in several instances (Koren et al., 2012; Liou et al., 2012; Ridaura et al., 2013; Turnbaugh et al., 2006; Vijay-Kumar et al., 2010). However, use of the term "dysbiotic" must be accompanied by the recognition that the

definition of a healthy microbiota that should serve as a frame of reference is still poorly defined. It is possible that the microbiota of a healthy Westerner is also dysbiotic, significantly increasing the risk of developing diseases characterized by excessive or inappropriate immune inflammatory responses.

Microbiota-Accessible Carbohydrates: Food for the Masses

Much of the carbon and energy for members of the microbiota originate from plant- and animal-derived dietary carbohydrates (Koropatkin et al., 2012; Salyers et al., 1977). These carbohydrates are composed of monosaccharides that are connected through numerous types of glycosidic linkages and, in some instances, further modified by chemical substituents like acetyl and sulfate groups. The variation in their chemical composition, solubility, and size differentiates these carbohydrates into a vast array of ecological niches. However, microbial competition within the gut is intense for metabolic access to the energy and carbon sequestered in these molecules. Residents of the microbiota are equipped with a specialized collection of enzymes such as glycoside hydrolases and polysaccharide lyases, which can break down complex carbohydrate linkages into consumable oligosaccharides or monosaccharides (Cantarel et al., 2009; Martens et al., 2011). A model microbiota containing ~160 species has in excess of 9,000 glycoside hydrolases and 2,000 polysaccharide lyases, which serve as the gateway to a complex microbial food web within the gut (El Kaoutari et al., 2013). When extrapolating to ~1,000 species, a single human gut microbiota may contain upward of 60,000 carbohydrate-degrading enzymes. The human genome, on the other hand, has

Box 1. Microbiota-Accessible Carbohydrates

MACs are carbohydrates that are metabolically available to gut microbes. MACs include carbohydrates that are dietary and resistant to degradation and absorption by the host, and they may be secreted by the host in the intestine (e.g., mucus) or produced by microbes within the intestine. Dietary MACs may come from a variety of sources including plants, animal tissue, or food-borne microbes, but must be metabolized by the microbiota. Much of the cellulose humans consume is not metabolized by gut microbes and does not qualify as a MAC (Chassard et al., 2010). The amount of dietary MACs present in a single food source differs for each individual, since which carbohydrates are metabolized depends upon the membership of each person's microbiota. The individuality of which carbohydrates qualify as MACs is illustrated by the presence of genes for the consumption of the algal polysaccharide porphyran in the microbiomes of Japanese individuals, rarely found in North American and European individuals (Hehemann et al., 2010, 2012). For those harboring a porphyran-degrading strain, porphyran would be a MAC; but for those without a microbiota adaptation to seaweed, porphyran would not be a MAC. Similarly, germ-free mice that lack a microbiota might consume a diet high in *potential* MACs, but none of the carbohydrates would be considered bona fide MACs, since they would transit the digestive tract unmetabolized by microbes. Alternatively, lack of dietary MACs results in a microbial community reliant upon endogenous host-derived MACs in the form of mucin glycans (Sonnenburg et al., 2005). Different host genotypes can influence the identity of MACs available to the microbiota in multiple ways. For example, host genotype can dictate alteration of mucus structures, such as the absence of alpha-1-2 fucose residues in the mucus of nonsecretor individuals that lack alpha-1-2-fucosyltransferase activity in the intestine (Kashyap et al., 2013b). Similarly, host genotype can determine how efficiently carbohydrates are digested and absorbed in the small intestine. For example, lactose becomes a substrate for the microbiota in people who are lactose intolerant, and therefore should be considered a MAC for these individuals. For nursing infants, dietary MACs in breast milk are known as human milk oligosaccharides (Bode, 2012; Marcobal et al., 2010, 2011). Therefore, the term "microbiota-accessible carbohydrate" contributes to a conceptual framework for investigating and discussing the amount of metabolic activity that a specific food or carbohydrate can be expected to produce within a given microbiota (Figure 1).

only a small number of glycoside hydrolases (~17) and no polysaccharide lyases that are involved in carbohydrate digestion within the gut.

Carbohydrates for gut microbe fermentation can come from a variety of sources including (1) dietary or host-derived animal glycans (2) synthesized by other microbes that are food-borne (e.g., the yeast cell wall) or a gut resident, and (3) dietary plant material, commonly referred to as dietary fiber, which is the most common fuel for the microbiota of many humans (Flint et al., 2012; Korpapatkin et al., 2012). The role of dietary fiber on human health has been discussed for decades. In the 1960s and 1970s, Denis Burkitt and Hugh Trowell documented the significantly larger intake of dietary fiber by Africans relative to Westerners and their coincident lack of Western diseases such as diabetes, heart disease, and colorectal cancer. Many of these diseases, including asthma, allergies, and inflammatory bowel diseases, appear in young Westerners and are therefore not simply a product of our modern extension of lifespan, but share a dysregulated immune system as a common basis. Burkitt reported that rural Africans passed stool that was up to five times greater by mass, had intestinal transit times that were more than twice as fast, and ate three to seven times more dietary fiber (60–140 g versus 20 g) than their Western counterparts (Burkitt et al., 1972). However, the mechanisms by which dietary fiber would exert positive health effects were unknown. Burkitt and others hypothesized that the increased transit time and stool size that accompanied a high-fiber diet may serve to dilute potentially hazardous microbial metabolic products and quickly remove them from the colonic lining (Burkitt and Trowell, 1977; Trowell and Burkitt, 1986). But recent studies are suggesting that the missing mechanistic explanation for the beneficial effects of dietary fiber may be largely attributed to fermentation by the microbiota.

In discussing carbohydrate substrates that fuel the colonic microbial ecosystem, dietary fiber is a problematic term commonly

employed for lack of a better option. The definition of dietary fiber has evolved over the past 60 years since the concept was first introduced (Hipsley, 1953; Trowell, 1976; Trowell et al., 1976; Trowell and Burkitt, 1987). Current use of this term is complicated by multiple definitions associated with different official organizations (Raninen et al., 2011). In addition, multiple laboratory tests may be used to determine "dietary fiber" content specified on nutritional labels, none of which provides an accurate measure of what the definitions specify. The most standard method of quantifying dietary fiber content neglects many types of carbohydrates that are destined for microbiota fermentation in the colon, like inulin, but includes noncarbohydrate entities like lignin. In addition, the term dietary fiber encompasses the carbohydrates that are fermentable by an individual's microbiota plus those that remain unfermented and serve a bulking role (Kashyap et al., 2013a). Attempts to use measurements of soluble versus insoluble fiber as approximations of fermentable versus nonfermentable, respectively, have proven inaccurate. Similar to "dietary fiber," the term "plant polysaccharides" is a common euphemism for "microbiota food," but this category excludes important nonplant carbohydrates and oligosaccharides.

When referring to the carbohydrates that can be metabolically used by gut microbes, we propose the term "microbiota-accessible carbohydrate" (MAC; see Box 1 for expanded discussion of MACs). MACs serve as selective agents, altering the composition of the microbiota, but also dictate the functionality and metabolic output. Multiple studies have shown that diet can serve as a selective potent force in reshaping the microbiota (David et al., 2014; Walker et al., 2011; Wu et al., 2011). Highly controlled experiments in mice have shown that the quantity and type of carbohydrates that feed the microbiota alter simplified and complex microbial communities (Faith et al., 2011; Kashyap et al., 2013a; Sonnenburg et al., 2010; Turnbaugh et al., 2009b). Humans eating controlled diets supplemented with

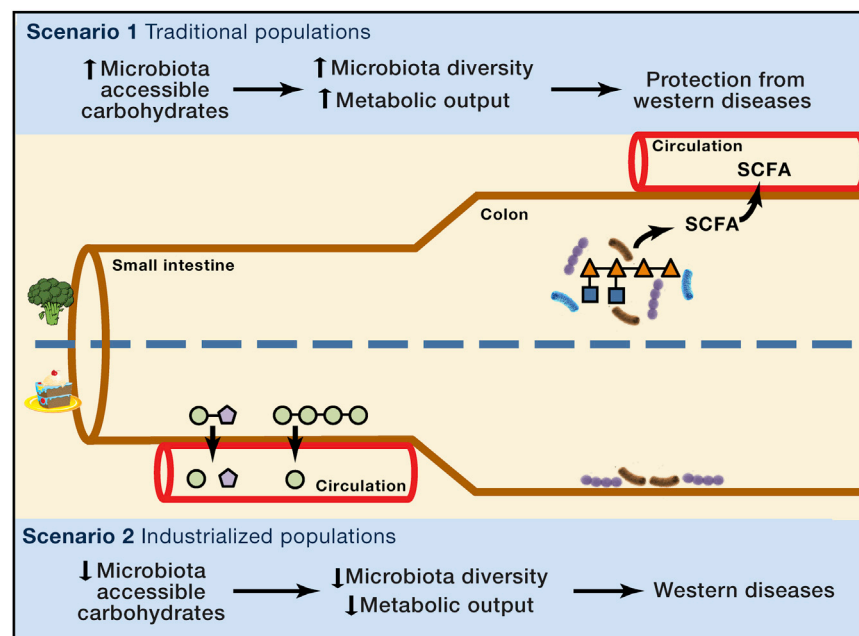


Figure 1. The Divergent Metabolic Scenarios of a High-MAC versus a Low-MAC Diet

Two scenarios represent a trade-off in how calories are absorbed by the host. In the first scenario, a high-MAC diet that has few simple sugars, the major contribution of carbohydrates to host metabolism is in the form of the SCFA fermentation end-products of the microbiota. In addition to calories, these molecules play diverse regulatory roles in human physiology, including protection from many Western diseases. In the second scenario, the low-MAC Western diet results not only in a loss of beneficial microbial metabolites such as SCFA, but also in selection of a distinct microbiota that may seem foreign to the host. Increased representation of mucus-utilizing microbes, decreased gut motility, and increased calories in the form of sugar and fat may synergize to cause Western diseases. MACs include carbohydrates from diet, host secretion (e.g., mucin glycans), or other resident microbes that serve as a metabolic input for members of the microbiota.

nonstarch polysaccharides or resistant starch, both with high levels of potential MACs, exhibit diet-induced alterations in microbiota composition (Walker et al., 2011). Due to the “gateway” role of primary fermenters within the colonic ecosystem, MACs can promote certain microbes either directly (those that consume a substrate) or indirectly (via crossfeeding interactions) (Fischbach and Sonnenburg, 2011). Despite a large gap in our understanding of how the diversity and quantity of dietary MACs influence host health, many studies are strengthening the links between MACs, microbiota diversity and metabolism, and host health.

MACs and Microbiota Diversity

Accumulating data suggest that host health is linked to dietary MAC-induced alterations in microbiota composition and diversity. A dietary intervention performed on 49 obese individuals showed an increase in microbiota gene richness when participants reduced energy intake and increased dietary fiber consumption for 6 weeks (Cotillard et al., 2013). In mice, reduction of dietary fiber has a direct effect on microbiota diversity and short-chain fatty acid production; the latter is an indication that indeed the dietary fiber includes MACs (Kashyap et al., 2013b; Trompette et al., 2014). Recently, a significant and reversible difference in microbiota composition was observed in humans that shifted to either a diet of all plants or one of all animal products, illustrating the impact diet has on microbiota composition (David et al., 2014). However, despite significant increases in dietary fiber (and presumably MAC) intake over the 4-day plant-based diet, an increase in microbiota diversity was not observed. Four days of increased dietary MACs may be an insufficient time period to for previously rare (undetectable) species to flourish or for environmentally acquired microbes to entrench. It remains to be determined whether the simplified Western microbiota has lost species that cannot be recovered upon increased dietary MACs.

Additional human studies have correlated increased dietary MAC consumption to microbiota composition, diversity, and health. The gut microbiota of children from a rural village in Burkina Faso revealed stark differences compared to a “healthy” Western cohort in Italy (De Filippo et al., 2010). In addition to disparity in represented phylotypes, relative to the Europeans, the African group showed (1) an altered ratio of the two dominant phyla of the human microbiota, with increased representation of Bacteroidetes relative to Firmicutes (the opposite of the Bacteroidetes depletion associated with obesity in several studies) (Ley et al., 2005, 2006; Turnbaugh et al., 2009a); (2) increased microbiota diversity; and (3) large increases of SCFA production including 4-fold more butyrate and propionate. These changes coincide with nearly twice the dietary fiber intake in the African group compared to the European group.

Our Ancient Microbiota

While the Burkina Faso study strengthened the link between diet and microbiota diversity, it also provided the first glimpse at what the human microbiota may have looked like during the Neolithic revolution in agriculture as humans embraced cultivated foods. An independent study of rural groups in Malawi and Venezuela similarly revealed greater bacterial diversity and stark differences in microbiota composition of nonmodern societies relative to Western groups (Figure 2). The microbiota from all three traditional agrarian groups in Malawi, Venezuela, and Burkina Faso were more similar to one another in microbiota diversity and SCFA production than to their Western counterparts (Yatsunenko et al., 2012). In addition to supporting the view that the modern microbiota deviates substantially from ancestral states, these findings point to the need to infer the microbiota at additional time points of human evolution, such as that of preagricultural humans.

Hunting and gathering was the lifestyle that dominated for the longest time span in our species existence. The Hadza, a group

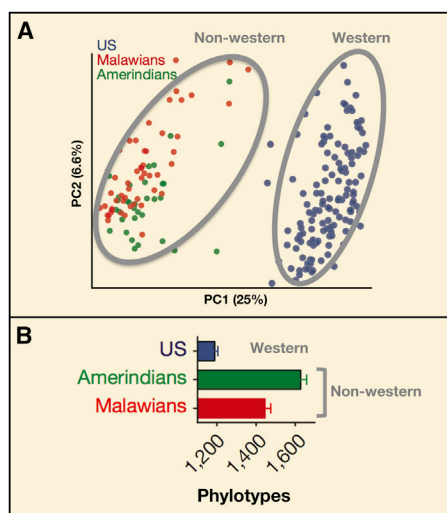


Figure 2. The Western Microbiota Diverges from That of Non-Western Populations

(A) Principle coordinate plot of gut microbiota composition in individuals (each dot represents one person's microbiota) from three populations based on Unifrac distances of the bacterial 16S rRNA profile. Distance between dots represents extent of compositional difference.

(B) Average number of bacterial phylotypes detected within the gut microbiota from three populations; error bars indicate SEM. Data reprinted with permission (Yatsuneneko et al., 2012).

of ~1,000 people in Tanzania, are the last full-time hunter-gatherers in Africa and represent one of the best modern approximations of preagricultural humans (Marlowe, 2010). A recent study comparing the microbiota of 27 Hadza to 16 Italians, who represent a Western cohort, showed many interesting differences, including a relative enrichment of the *Prevotella* genus, the presence of *Treponema* species, and the lack of *Bifidobacterium* (Schnorr et al., 2014). Perhaps their most significant finding is the much larger extent of bacterial diversity in the foraging microbiota compared to the industrialized group.

Interpretation of these studies comes with important caveats. Most studies focus on the bacterial component of the gut microbiota, excluding important archaea, eukaryotes, and viruses. All of these rural populations are composed of modern people that approximate, but are not exact representations of, a human ancestral state. Furthermore, it is almost certain that there was no single "ancestral state" for any given time in human evolution. As humans radiated across the planet, diverse lifestyles, diets, locations, and genetic differences likely resulted in microbiota variation within populations; it is certain that even gradients of transculturation exist within each indigenous cohort. So additional microbiota data, coupled with meta-data about diet and lifestyle from these and other traditional populations, will be required to place these initial findings into context. Paleogenomics offer an alternative avenue to elucidate, rather than infer, microbiotas of the past (Tito et al., 2012). The major dietary shifts occurring between the hunter-gatherer lifestyle, early Neolithic farming, and more recently during the Industrial Revolution are reflected in changes in microbial membership within dental tartar of European skeletons throughout these periods (Adler et al., 2013). Notably, the transition to our modern lifestyle has been accompanied by the

appearance of cariogenic bacteria and markedly reduced oral microbiota diversity.

Microbiota Metabolism and Host Health

Two independent studies of individuals in Europe have illustrated that microbiome diversity is linked to human health (Cotillard et al., 2013; Le Chatelier et al., 2013). The 341 combined participants from both studies were comprised of obese, overweight, and healthy individuals and exhibited a bimodal distribution of microbiota gene content: some individuals exhibited low gene count (LGC) and others high gene count (HGC). The LGC group was more insulin resistant and had higher fasting triglycerides, higher LDL cholesterol, and higher markers of inflammation relative to the HGC group. Dietary intervention of 49 obese or overweight individuals (18 from the LGC group and 27 from the HGC group) resulted in loss of fat mass and was accompanied by improvements in clinical markers such as lipid and insulin values, insulin resistance, and measures of inflammation for both the LGC and HGC groups (Cotillard et al., 2013). However, improvements in these clinical markers were most pronounced for the HGC group, indicating that microbiome richness was a predictor of response to diet-induced improvements. Dietary intervention did result in an increase in gene richness in the LGC cohort that approached but still remained significantly lower than the HGC. Together these independent studies indicate that metabolic disease clinical phenotypes are associated with gene content diversity of the gut microbiota and that diet can directly impact this diversity.

The mechanisms underlying the link between microbiota diversity and health are still unknown, but SCFAs appear to be one likely mediator. It is possible that microbiota diversity is an intermediate correlate of high-MAC diets and that fermentation end-products absorbed into circulation are important effectors of host physiology. The short-chain fatty acids, acetate, propionate, and butyrate, are common end-products of carbohydrate-fermenting microbes in the distal gut (Wong et al., 2006), and several factors, including dietary substrates, can affect the relative ratios and concentrations (Macfarlane and Macfarlane, 2003; Topping and Clifton, 2001). SCFAs are absorbed by the host and serve as calories salvaged from otherwise inaccessible carbohydrates, but they also play diverse regulatory roles including regulating histone acetylation and signaling through G protein coupled receptors (Bergman, 1990; Brown et al., 2003; Davie, 2003). Recently several groups have linked microbiota-generated SCFAs to anti-inflammatory effects. SCFAs increase the pool of regulatory T cells in the gut, and MAC-dependent SCFAs produced by the gut microbiota are protective of allergic airway inflammation in a mouse model (Arpaia et al., 2013; Furusawa et al., 2013; Smith et al., 2013; Trompette et al., 2014). Multiple studies indicate a dependence upon fatty-acid, G protein-coupled receptors (GPR41 or GPR43).

SCFAs regulate energy homeostasis, a role to which the beneficial metabolic effects of fiber have been attributed (Layden et al., 2013). Butyrate appears to broadly influence metabolism and circulating propionate signals via the afferent nervous system to influence gluconeogenesis in the intestine, as well as results in improved metabolic profiles and reduced weight gain (De Vadder et al., 2014; Gao et al., 2009; Lin et al., 2012).

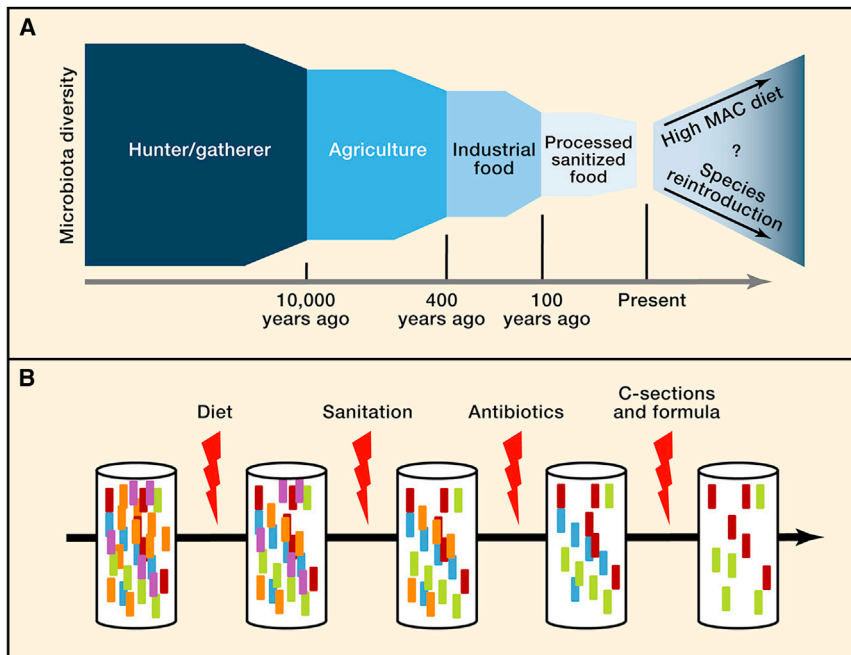


Figure 3. The Multiple-Hit Hypothesis for How the Microbiota of Industrialized Societies Has Lost Diversity over Time

(A) Microbiota diversity was likely altered at multiple stages of human evolution. As diversity and quantity of dietary MACs decreased with agriculture, industrialized food production, and processed food, the model reflects data that indicate a corresponding decrease in microbiota diversity. (B) While diet is likely a key mediator of microbiota diversity, additional technological and medical leaps, while providing solutions for important problems such as infectious disease, have likely served as insults to the microbiota. These multiple hits have prevented the maintenance of microbiota diversity over recent generations.

crobial part of our biology could easily adjust to day-to-day or season-to-season variation in available food. Considering the remarkable resilience of the gut community to short-term perturbation, it is likely that microbiota adaptation is largely reversible on short time scales (David et al., 2014; Dethlefsen and Relman, 2010; Lee et al., 2013). However, the ability of the microbiota to accommodate dietary change may be deleterious to its own maintenance and ultimately to our health in the context of MAC deprivation that has occurred over generations in industrialized countries. In addition to the loss of microbial species with which we have coevolved, the lack of MACs required to fuel this community results in profound functional changes, such as decreases in SCFA production.

Additional studies have linked dietary fiber and the resulting SCFAs to protection from diet-induced obesity (Cani et al., 2007; Lin et al., 2012; Neyrinck et al., 2012; Zhou et al., 2009). It remains to be determined how dietary MAC consumption influences the thousands of microbiota-produced metabolites and the resulting influence on host biology (Marcobal et al., 2013; Nicholson et al., 2012; Russell et al., 2011, 2013).

Effects from decreased SCFA output on the low-MAC Western diet may be compounded by changes in microbiota localization and barrier disruption in the distal gut. The paucity of fermentable carbohydrates selects for a community of microbes that are adept at eating host-derived MACs found in the intestinal mucus layer. Consumption of host mucins by many gut species serves as a consistent endogenous source of energy for the microbiota in the absence of dietary polysaccharides (Sonnenburg et al., 2005). The long-term effects of low dietary MACs on the gut mucus depletion, barrier function, and inflammation have not been studied in detail; however, genetic mucus depletion in mice results in microbiota-induced inflammation and ultimately colitis (Johansson et al., 2014; Van der Sluis et al., 2006).

The Future: Looking to the Past

Whether the ancient microbiota is a better microbiota is debatable; however, at least two arguments support this idea: (1) our human genome lacks the plasticity that our microbiota possesses and therefore has not been able to keep pace with the recent profound changes brought on by our modern lifestyle, such as the consequences of a diet very low in microbiota-accessible carbohydrates (e.g., low diversity microbiota, less SCFA production); and (2) traditional societies typically have much lower rates of Western diseases (Wirsing, 1985).

The plasticity of our microbiota in response to diet was likely highly adaptive in an ancient environment. In delegating part of our digestion and calorie harvest to our gut residents, the mi-

crobial part of our biology could easily adjust to day-to-day or season-to-season variation in available food. Considering the remarkable resilience of the gut community to short-term perturbation, it is likely that microbiota adaptation is largely reversible on short time scales (David et al., 2014; Dethlefsen and Relman, 2010; Lee et al., 2013). However, the ability of the microbiota to accommodate dietary change may be deleterious to its own maintenance and ultimately to our health in the context of MAC deprivation that has occurred over generations in industrialized countries. In addition to the loss of microbial species with which we have coevolved, the lack of MACs required to fuel this community results in profound functional changes, such as decreases in SCFA production.

Undoubtedly, sudden changes in human diet throughout evolution resulting from major lifestyle changes (e.g., cooking, hunting with weapons, and farming) were accompanied by fundamental shifts in microbiota composition and function (Figure 3A). These new configurations of the microbiota likely introduced suboptimal interactions with many aspects of human biology; however, such interactions would be expected to optimize over millennia. It is possible that the new microbiota configuration resulting from modern diet and lifestyle presents incompatibilities with our human biology, the ill effects of which contribute significantly to many modern diseases (Figure 3B) (Blaser and Falkow, 2009).

Significant questions remain unanswered to address how large a role the Western microbiota plays in modern diseases. How important is the loss of coevolved microbial species versus the loss of microbial metabolites? Would a more sensible diet that increases MAC content be sufficient to recover microbiota diversity, or have species been irreversibly lost? Does imposing a high-MAC diet on a Western microbiota present new incompatibilities and potential unanticipated negative health consequences? We must be aware that ongoing dissemination of the industrialized lifestyle and processed foods to the most remote areas of the globe will be accompanied by a series of microbial extinction events similar to that which has left the biodiversity of the Western microbiota decimated. While imposing poorer health on populations as they modernize, the global eradication of critical microbiota members will likely have far-reaching importance. These remaining untouched populations may

represent valuable repositories of important taxa that have been lost from Western populations. Collection and preservation of these microbes followed by their deliberate reintroduction supported by a diet rich in the MACs that sustain them might be the best hope of rewilding the Western microbiota.

ACKNOWLEDGMENTS

We thank members of the Sonnenburg lab and Jeff Leach for valuable discussions and Sara Fisher for editing this manuscript. This research was supported by R01-DK085025 and DP2-OD006515. J.L.S. holds an Investigators in the Pathogenesis of Infectious Disease Award from the Burroughs Wellcome Fund.

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